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TRANSPORT OF INORGANIC SULFATE BY THE RENAL TUBULES

BY

FREDRIK BERGLUND

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From the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden

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FREDRIK BERGLUND

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BERGLUND, F., C.-G. HELANDER and R. B. HOWE, Inorganic sulfate and thiosulfate: transport and competition in the renal tubules of the dog. *Amer. J. Physiol.* 1960. *198*. 586—594.

BERGLUND, F. and W. D. LOTSPEICH, Renal tubular reabsorption of inorganic sulfate in the dog, as affected by glomerular filtration rate and sodium chloride. *Amer. J. Physiol.* 1956 a. *185*. 533—538.

BERGLUND, F. and W. D. LOTSPEICH, Effect of various amino acids on the renal tubular reabsorption of inorganic sulfate in the dog. *Amer. J. Physiol.* 1956 b. *185*. 539—542.

COHEN, J. J., F. BERGLUND and W. D. LOTSPEICH, Renal tubular reabsorption of acetoacetate, inorganic sulfate and inorganic phosphate in the dog as affected by glucose and phlorizin. *Amer. J. Physiol.* 1956. *184*. 91—96.

COHEN, J. J., F. BERGLUND and W. D. LOTSPEICH, Interrelations during renal tubular reabsorption in the dog among several anions showing a sensitivity to glucose and phlorizin. *Amer. J. Physiol.* 1957. *189*. 331—338.

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Introduction

This presentation will summarize experimental work done on the transport of inorganic sulfate by the renal tubules. This transport takes opposite directions in different animals. In marine fish, sulfate is secreted by the tubules, i.e. transferred from blood to tubular urine. In mammals, sulfate is reabsorbed from the tubular urine. Both secretion and reabsorption are limited by a "**transfer maximum**" or **Tm**. There is also competition with thiosulfate in both directions. Thus, secretion and reabsorption of inorganic sulfate seem to be basically the same process.

The initial section on comparative physiology will direct attention to the important role of the kidneys for regulation of sulfate levels in the animal organism. The remaining sections deal specifically with the renal tubular secretion or reabsorption of inorganic sulfate. Effects of other substances, which are also secreted or reabsorbed by the tubules, will be emphasized.

Comparative physiology background. Osmotic and ionic relations

Invertebrates

Among the invertebrates, osmo-regulation and ionic regulation of the internal environment have developed partially independent of one another. Most marine invertebrates are isotonic with sea water, although some crustaceans and annelids are hypotonic (PROSSER et al. 1950). (Osmolal concentration of sea water is around 1 osm/kg H_2O, with a freezing point depression $\Delta_{f.p.}$ = = 1.8° C) — Magnesium and sulfate levels are usually lower in marine invertebrates than in sea water, which holds around 50 mmoles/l of Mg^{++} and 30 mmoles/l of $SO_4^=$. Coelenterates and echinoderms lack special excretory organs, and show the least ionic regulation. The crustacea, in contrast, possess "antennary glands", which secrete Mg^{++} and $SO_4^=$ (urine/plasma concentration ratios as high as 3.9), thereby lowering the plasma concentrations to 10—20 % of sea water levels.

In fresh water and terrestrial invertebrates, the osmolality shows considerable variation, but is always lower than in sea water. The concentration of inorganic sulfate in the body fluids is less than 2 μmoles/ml (PROSSER et al. 1950).

Vertebrates

Osmo-regulation in vertebrates is effected by kidneys, gills (in fish), skin (especially in amphibians), cloaca (reptiles and birds) as well as by special "salt glands" (elasmobranchs, reptiles and birds).

The osmolal concentration of vertebrate blood shows values between 0.24 and 0.65 osm/kg H_2O (PROSSER et al. 1950). Thus the blood is hypotonic to sea water. The elasmobranchs form an important exception. Their blood is slightly hypertonic to the medium, partly due to high concentrations in the blood of urea (up to 0.44 M) (SCHRÖDER 1890) and trimethylamine oxide (0.1 M) (HOPPE-SEYLER 1930).

From the viewpoint of ionic regulation, vertebrates are characterized by consistently low concentrations of calcium, magnesium and sulfate. In marine fish, ingested sea water is a rich source of these ions (SMITH 1930); they are absorbed from the gut and subsequently excreted in the urine. — The ratio between renal and extra-renal excretion of inorganic ions after alimentary absorption has been studied in the American eel (Anguilla rostrata) by Smith. Univalent ions (Na^+, K^+, Cl^-) were excreted mainly by an extra-renal route (gills). When $CaCl_2$, $MgCl_2$ or Na_2SO_4 was injected into the gut, the divalent ions were excreted to more than 90 % by the kidneys; Ca^{++} and Mg^{++} taking with them Cl^- into the urine, $SO_4^=$ taking along Na^+.

Table 1. Concentration of divalent ions in sea water, and in plasma and urine of marine fish

Species	Ca^{++} μmoles/ml	Mg^{++} μmoles/ml	$SO_4^=$ μmoles/ml	Reference
Sea water	10	47—53	25—35	Prosser 1950
Lophius americanus (goosefish).				
Plasma	3.5	1.0	1.2	Marshall and
Urine	12	95	44—180	Grafflin 1928
Plasma	1.0—3.9	0.4—8.2	0.4—2.8	Forster and
Urine	0.5—30	3—140	17—75	Berglund 1956
Myoxocephalus scorpius (shorthorn sculpin).				
Urine		72—214	28—120	Pitts 1934
Myoxocephalus octodecimspinosus (longhorn sculpin).				
Urine	8—39	76—142	32—61	Smith 1930
Urine		36—194	6—85	Pitts 1934
Anguilla rostrata (American eel).				
in sea water Urine	7—20	75—167	34—125	Smith 1930
in fresh water Urine		0.7	1.3	
Raja laevis (barndoor skate)				
Plasma		2.4	Trace	Marshall and
Urine		65	44	Grafflin 1928
Squalus acanthias (spiny dogfish)				Berglund and
Plasma			0.4—1.4	Forster, unpub-
Urine			20—86	lished, 1954

The concentration of Ca^{++}, Mg^{++} and $SO_4^=$ in urine of marine fish is higher than in plasma, and often higher than in sea water (table 1). This is true in teleosts with aglomerular or predominantly aglomerular renal tubules (Lophius and Myoxocephalus scorpius), or with completely glomerular kidneys (Myoxocephalus octodecimspinosus and Anguilla); it is also true in elasmobranchs (Raja and Squalus). It is evident that the inorganic divalent ions, Ca^{++}, Mg^{++} and $SO_4^=$, are effectively excreted by the kidneys of marine fish. In contrast, when the eel is transferred to fresh water, its urine contains very little Mg^{++} and $SO_4^=$.

Table 2. Ionic concentrations in excreta of herring gull, Larus argentatus

Source	Na^+	K^+	Cl^-	$SO_4^=$
	µmoles/ml			
Nasal secretion, 5 minute periods	718 724 720	24 24 32	700 854 729	0.3 0.1 0.4
Cloacal urine	19	18	126	77
Urine + Feces	11	26	67	125
Sea water	437	9	488	28

Body weight 835 g. 47 ml sea water administrated by stomach tube before experiment (K. SCHMIDT-NIELSEN, unpublished experiment, 1957. Sulfate analyzed by R. B. HOWE, by benzidine precipitation).

Marine birds and reptiles may also ingest sea water. Under such conditions the "salt glands" play an important role for the osmo-regulation in various species of birds (SCHMIDT-NIELSEN, JÖRGENSEN and OSAKI 1958) and reptiles (SCHMIDT-NIELSEN and FÄNGE 1958). Sodium chloride is excreted in the "nasal secretion" by these glands in a concentration considerable higher than in sea water. The experiment shown in table 2 shows that the "nasal secretion" of the herring gull, after oral administration of sea water, contained Na^+, Cl^- and K^+, but practically no $SO_4^=$. The latter ion seemed to be freely excreted in the urine, in which its concentration was more than twice as high as in sea water.

In fresh water and terrestrial vertebrates, inorganic sulfate is mainly derived from the metabolism of sulfur-containing amino acids, i.e. cystine, cysteine and methionine. The concentration of inorganic sulfate in plasma is around 1.3 µmoles/ml in the dog (REED and DENIS 1927, GOUDSMIT, POWER and BOLLMAN 1939), and around 0.35 µmoles/ml in man.

At least 20 papers on inorganic sulfate in human plasma or serum (with reasonable documentation of methods and results) have been published during the past 40 years. The mean values vary from 0.10 (CUTHBERTSON and THOMPSETT 1931) to 0.62 µmoles/ml (WATCHORN and MCCANCE 1935). A thorough study of sulfate in human plasma was presented by ØLLGAARD (1937); he analyzed sulfate by precipitation with benzidine from trichloroacetic acid filtrates of citrate plasma. The method was checked by recovery studies on sulfate-free (dialyzed) plasma. In 100 plasmas, obtained from hospital patients in the morning, the concentration of inorganic sulfate averaged 0.35 µmoles/ml (table 3). The sulfate levels were around 30 % higher in the evening in subjects on a normal diet; a low protein diet abolished this rise. In patients with diabetes mellitus (diet not specified), the sulfate levels were somewhat lower (table 3);

Table 3. Concentration of inorganic sulfate in plasma of man (ØLLGAARD 1937)

Subjects Conditions	Number of subjects	Mean ± stand. dev.	Range
		µmoles/ml plasma	
Patients, no renal disease, no diab. mellitus, 12 hours fasting	100	0.35 ± 0.049	0.25—0.47
Diabetes mellitus, insulin treatment	19	0.29 ± 0.054	0.17—0.40
	Normal versus Diabetes $P < 0.001$		

no relation to blood glucose level was found. — In a recent study, using a turbidimetric barium method, BERGLUND and SÖRBO (1960) obtained an average level of inorganic sulfate of 0.33 µmoles/ml serum in 10 normal subjects. The method was checked by recovery studies on dialyzed serum and by simultaneous analysis with benzidine.

In cerebrospinal fluid, the concentration of inorganic sulfate is less than 1/3 of that in plasma; this applies both at normal plasma sulfate levels, and at elevated levels in patients with chronic nephritis (WATCHORN and MCCANCE 1935, ØLLGAARD 1937). In patients with meningitis, the sulfate level in cerebrospinal fluid is almost as high as in plasma (WATCHORN and MCCANCE 1935).

Measurement of sulfate transport in the renal tubules

The kidneys play a dominant role for the excretion of sulfate in vertebrates. The amount excreted in the urine is the net result of three processes: 1. Filtration in the glomeruli; 2. Secretion by the tubules; 3. Reabsorption by the tubules. This review deals mainly with the quantitative aspects of the latter two processes.

Net secretion, or net reabsorption, of sulfate by the renal tubules is measured by the difference between the amount filtered in the glomeruli and the amount excreted in the urine. Thus:

$$\text{Reabsorbed } SO_4 = \text{Filtered } SO_4 - \text{Urine } SO_4$$

or

$$\text{Secreted } SO_4 = \text{Urine } SO_4 - \text{Filtered } SO_4$$

In both cases:

$$\text{Filtered } SO_4 = \text{Glomerular filtration rate (GFR)} \times \text{Plasma } SO_4$$

GFR is measured by the clearance (C) of any substance that is completely filterable in the glomeruli, and neither secreted nor reabsorbed by the tubules. The clearance of a substance X is obtained by dividing the amount appearing in the urine per unit time by the plasma concentration, as expressed in the formula: $C_X = \frac{U_X \cdot V}{P_X}$, in which U_X = concentration of X in the urine, V = urine volume per unit time, and P_X = concentration of X in plasma.

An early attempt to measure GFR in the rabbit was made by Mayrs and Watt (1922), using the clearance of exogenous sulfate at sulfate levels between 15 and 53 μmoles/ml plasma. Simultaneously renal blood flow and hematocrit were measured, and renal plasma flow was calculated from these values. "Filtration fraction" was finally obtained by dividing sulfate clearance (GFR) by renal plasma flow. In 11 experiments, the filtration fraction averaged 19 %.

Mayrs and Watt had chosen sulfate to measure GFR, because of the high urine/plasma concentration ratio for sulfate compared with that of urea. They reasoned that urea was reabsorbed by the tubules, whereas sulfate probably was not.

Two decades later, Schou (1944) made simultaneous measurements of sulfate and creatinine clearances in rabbits (creatinine clearance equals GFR in this species (Kaplan and Smith 1935)). At normal plasma levels of sulfate (< 2.5 μmoles/ml), the sulfate/creatinine clearance ratio was less than 0.3; at sulfate levels between 30 and 60 μmoles/ml plasma, the two clearances were equal. This meant that Mayrs and Watt actually were measuring glomerular filtration rate in at least 5 of their 11 experiments.

Since 1922 various substances have been proposed for the measurement of GFR. Some of these have later been shown to be reabsorbed by the tubules, e.g. xylose (SHANNON and SMITH 1935) and sulfate (see page 15). Others were shown to be secreted in some species (creatinine) (SHANNON 1935), and some others may be both secreted and reabsorbed (thiosulfate, creatinine) (BUCHT 1949). The magnitude of error in using these substances for measuring GFR depends to a high degree on the plasma level used. — Finally, there are substances which seem to be neither secreted nor reabsorbed by the renal tubules, namely the ferrocyanide ion (except in man), inulin, polyethylene glycol (mol-weight $< 6\,000$) (see Smith 1951) and dextran (mol-weight $< 6\,000$—$7\,000$) (WALLENIUS 1954).

In the dog, exogenous creatinine clearance is generally used instead of inulin clearance. These two clearances are practically identical, except at extremely low GFR (LADD, LIDDLE and GAGNON 1956).

Filterable fraction of sulfate in plasma. The filtered load of sulfate equals GFR $\times$ Plasma SO_4, only if no binding of sulfate to plasma protein occurs. The filterability of sulfate has been investigated in heparin plasma of the dog by GOUDSMIT, POWER and BOLLMAN (1939) (5 samples), and in "normal" human serum by HAYMAN and JOHNSTON (1932) (3 samples), and WATCHORN and MCCANCE (1935) (17 samples). The concentration of sulfate in ultrafiltrates was compared with that of serum or plasma, using trichloroacetic acid filtrates, and benzidine precipitation methods for the sulfate analysis. In neither series was there any consistent difference in concentration between ultrafiltrates and serum.

WATCHORN and MCCANCE (1935) also made ultrafiltrates from patients with uremia and elevated plasma sulfate levels. Sera from 3 patients contained 0.94, 6.0 and 7.6 µmoles SO_4/ml; the corresponding ultrafiltrates held 1.03, 6.0 and 8.7 µmoles/ml.

It may be concluded that inorganic sulfate in plasma is completely ultrafilterable.

Tubular secretion of inoganic sulfate in marine fish

Renal excretion of inorganic divalent ions has been studied in detail in the aglomerular teleost, Lophius americanus (BERGLUND and FORSTER 1958), in which urine is formed only by tubular activity. Maximum rates of excretion (Tm) were reached for magnesium, sulfate and thiosulfate, when the plasma level of any of these ions was raised by intravenous injections. Injection of magnesium chloride markedly depressed calcium excretion; sodium thiosulfate similarly depressed the excretion of sulfate. The results suggested one transport system for inorganic divalent cations (Mg^{++} and Ca^{++}), and one for inorganic divalent anions ($SO_4^=$ and $S_2O_3^=$).

Probenecid, p-(dipropylsulfamyl)-benzoic acid, had no effect on the excretion of the inorganic ions, but Carinamide, p-(benzylsulfonamido)-benzoic acid, depressed the excretion of sulfate and thiosulfate. This was expected from earlier findings in man (BUCHT 1949) and in the dog (FOULKS et al. 1952).

Table 4. Carinamide and probenecid in plasma and urine of Lophius piscatorius

Time	Urine flow	Drug	
		Plasma	Urine
Hours	ml/kg/hr	mg/ml	mg/ml
Lophius A. 3.2 kg. Caught by beam trawl.			
0	9 ml 1 % *carinamide* i.m.		
0—3	0.44	0.05	0.55
3—9	0.83	0.03	0.60
9	5 ml 1 % *carinamide* i.v.		
9—13	1.12	0.05	0.70
13—19	1.37	0.04	0.63
Lophius C. 2.1 kg. Caught by otter trawl.			
0	10 ml 1 % *probenecid* i.m.		
0—3	0.54	0.11	0.05
3—8	0.96	0.08	0.08
8	5 ml 1 % *probenecid* i.v.		
8—13	1.22	0.12	0.07
13—18	1.00	0.11	0.08

Experiments done at Kristineberg Marine Biological Laboratory, Fiskebäckskil, Sweden, 1958. Carinamide and probenecid were analyzed by the method of BRODIE, LEVY and BERNSTEIN (1947), reading the absorbancies at 280 mμ and 242.5 mμ respectively. (BERGLUND, F. and R. B. HOWE, unpublished).

The difference between carinamide and probenecid on the sulfate-thiosulfate system is of interest, because these two drugs have an identical inhibitory effect on the tubular excretion of penicillin (BEYER et al. 1951). In an attempt to find an explanation for the difference, the renal excretion of the two drugs was looked into. Earlier work in the dog indicated that carinamide was secreted by the tubules (EARLE and BRODIE 1947), or both secreted and reabsorbed, the secretion being markedly depressed by p-aminohippurate (BEYER et al. 1949). Probenecid, on the other hand, was evidently only reabsorbed (BEYER et al. 1951). Extensive binding of both drugs to plasma proteins rendered some uncertainty to the interpretation of the data obtained in the dog. The excretion of carinamide and probenecid was therefore studied in Lophius piscatorius (the East Atlantic variety of Lophius, see BIGELOW and SCHROEDER 1953). Carinamide was excreted in the urine, but probenecid was not (table 4) (BERGLUND and HOWE, unpublished 1958). SPERBER (1954) has also reported tubular secretion of carinamide in the hen. Thus, among vertebrates, carinamide is secreted by the tubules, but probenecid is evidently not. It is possible, therefore, that carinamide, during its transport through the tubule cell, inhibits the secretion of thiosulfate and sulfate at a site which is not reached by probenecid.

As glycine had been shown to depress the tubular reabsorption of sulfate in the dog (BERGLUND and LOTSPEICH 1956 b), its effect was tested in Lophius (BERGLUND and FORSTER 1958). The excretion of sulfate was not affected. Glycine itself was not actively transported, but seemed to diffuse freely into the urine.

The high urine/plasma concentration ratios for sulfate in other marine teleosts (table 1) indicates that tubular secretion of sulfate occurs also in these species. This is most certainly true for the shorthorn ("daddy") sculpin, with predominantly aglomerular renal tubules.

The renal excretion of inorganic sulfate has also been studied in the spiny dogfish, Squalus acanthias (table 5) (BERGLUND and FORSTER, unpublished 1954). As seen from the last column, there was tubular secretion of sulfate,

*Table 5. Experiment showing tubular secretion of inorganic sulfate in the spiny dogfish, Squalus acanthias. (*BERGLUND *and* FORSTER, *unpublished, 1954)*

Time	Urine flow	Inulin clearance	SULFATE				
			Plasma	Urine	Filtered	Excreted	Secreted
Hours	ml/kg/24 hrs		µmoles/ml		µmoles/kg/24 hrs		
	Dogfish 2 1.7 kg						
0—4	24.2	56	.45	22.7	25	550	525
4—9½	24.4	80	.45	20.5	36	500	464
9½—13	29.1	101	.40	21.1	40	615	575
13—22	13.7	56	.42	37.8	24	518	494

which accounted for 94 % of the sulfate in the urine. In four other dogfish, the sulfate secreted by the tubules constituted more than 90 % of the total urinary sulfate in three, and around 83 % in one fish.

Thus, in a number of marine fish that have been examined (teleosts and elasmobranchs), the renal tubules secrete inorganic sulfate from the blood to the urine. In Lophius the secretion is inhibited by thiosulfate, probably by a competitive mechanism, and by carinamide.

Absence of tubular secretion of inorganic sulfate in the frog

YOSHIDA (1924) studied the renal handling of various substances in male frogs (Rana esculenta) with the "double perfusion technique", originally designed by Nussbaum. The kidneys were perfused with a modified Ringer solution, through the Aorta descendens (perfusion pressure 24 cm H_2O) and through the Vena abdominalis (perfusion pressure 10—12 cm H_2O); the latter fluid reached the kidneys via the renal-portal veins, and presumably perfused the peritubular capillaries without traversing the glomeruli.

In all experiments, there was much less NaCl in the urine than in the perfusing fluid. When 0.6 % Na_2SO_4 was added to the Aorta-fluid, the urine contained 0.7—1.0 % Na_2SO_4. With 0.6 % Na_2SO_4 in the Vena abdominalis fluid, the urine contained only 0.06—0.18 % Na_2SO_4. Yoshida concluded that the tubules reabsorbed filtered sodium chloride and also water, but were practically impermeable for the movement of sulfate in both directions. — His experiments seem to exclude tubular secretion of sulfate, but do not exclude reabsorption by the tubules.

Tubular reabsorption of inorganic sulfate in mammals

At normal plasma levels, the renal clearance of inorganic sulfate is only a fraction ($< 30\ \%$) of the glomerular filtration rate in a number of mammals, namely in man (HAYMAN and JOHNSTON 1932), in the dog (GOUDSMIT, POWER and BOLLMAN 1939), in the rabbit (SCHOU 1944), and in the goat (unpublished). When the plasma levels of sulfate are elevated, the clearance of sulfate approaches the glomerular filtration rate in man, the dog and the rabbit (not tested in the goat). Clearly, inorganic sulfate must be reabsorbed from the tubular urine, to a limited extent. The magnitude and the characteristics of this reabsorption have been most thoroughly studied in the dog.

Transfer maximum in the dog

The study by LOTSPEICH (1947) showed that in female dogs sulfate is almost completely reabsorbed from the tubular urine at normal plasma levels. The reabsorption curve obtained by Lotspeich is reproduced in fig. 1. The filtered load of sulfate is represented by the dashed line, the amount excreted in the

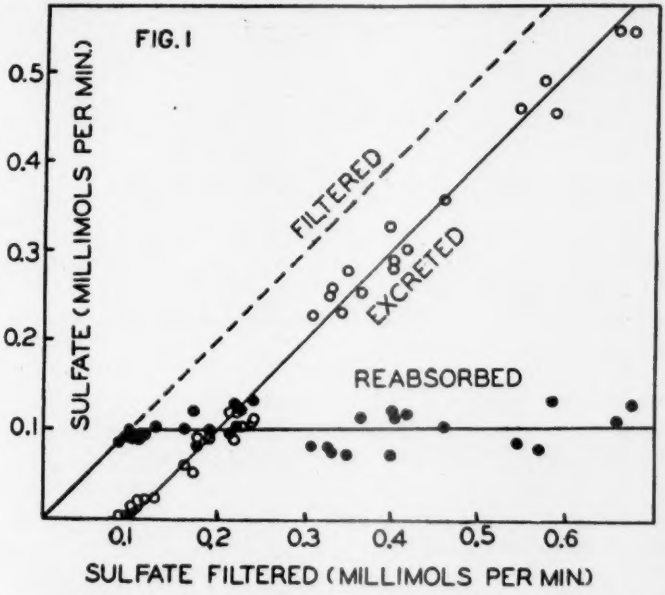

Fig. 1. Quantities of inorganic sulfate excreted and reabsorbed as a function of the quantity filtered (From LOTSPEICH 1947).

urine by the line through the circles. The vertical distance between these two lines represents the amount reabsorbed, and is shown by the horizontal line through the dots. It is evident that the sulfate reabsorption exhibits a "transfer maximum" (Tm).

It may also be seen that just a slight elevation of the plasma level above normal sufficed to saturate the reabsorption mechanism. It is therefore likely that the plasma level of sulfate is regulated by the kidneys, as is the case with sodium, potassium, chloride, bicarbonate and creatinine. This is in contrast to the regulation of glucose and phosphate in plasma; the concentration of these substances is normally regulated through the release or deposition in body stores, and only at markedly elevated plasma levels do the kidneys play a significant role.

Possibility of simultaneous secretion

The typical reabsorption Tm curve does not exclude simultaneous tubular secretion of inorganic sulfate. Fig. 2 illustrates this, for any substance X.

Line a represents the urinary excretion of X, if it is filtered only.

For *line ba*, the following assumptions were made:

1. Secretion occurs in the tubule at a site proximal to reabsorption, or through identical cells. Thus any substance that has been secreted might later be reabsorbed.
2. GFR = 100 ml/min. Renal plasma flow = 500 ml/min. Thus 500 — 100 = = 400 ml plasma/min perfuse the peritubular capillaries.
3. Reabsorption Tm = Secretion Tm = 100 μmoles/min.

Then, at low plasma levels (below 0.2 μmoles/ml), X is filtered in the glomeruli and secreted by the tubules, and finally completely reabsorbed; no X appears in the urine.

At a plasma level of 0.2 μmoles/ml, reabsorption Tm will be saturated, because

$$\begin{aligned} &\text{Filtered X} = 100 \times 0.2 = 20 \text{ μmoles/min} \\ &\text{Secreted X} = 400 \times 0.2 = 80 \text{ μmoles/min} \\ &\text{Reabsorbed X} = 20 + 80 = 100 \text{ μmoles/min} = \text{Reabsorption Tm} \end{aligned}$$

Urinary excretion then rapidly rises *(line b)*.

At a plasma level of 0.25 μmoles/ml, secretion Tm becomes saturated, and *line b* intercepts *line a*, as

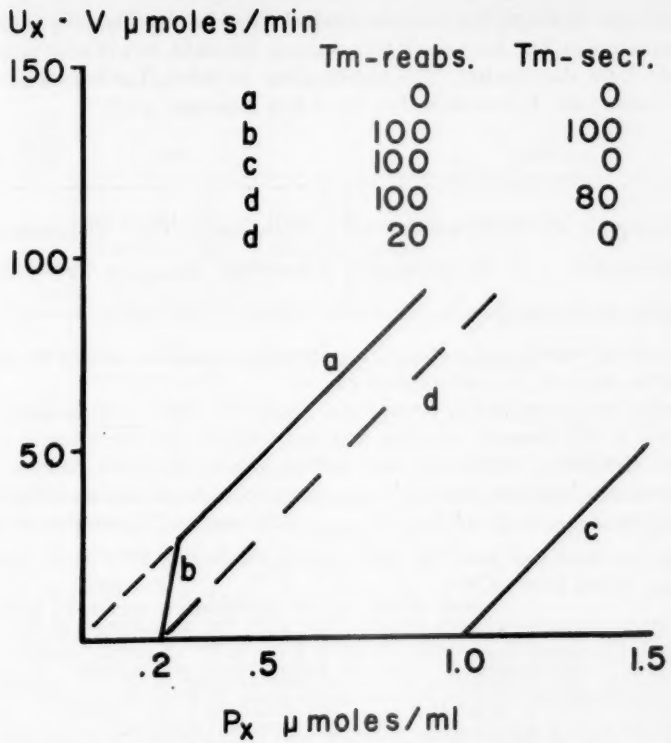

Fig. 2. Urinary excretion of any substance X, which is both secreted and reabsorbed. Secretion proximal to, or at same site as, reabsorption. GFR = 100 ml/min. Renal plasma flow = 500 ml/min. Note two possible interpretations of *line d*.

Filtered X = 100 × 0.25 = 25 µmoles/min
Secreted X = 400 × 0.25 = 100 µmoles/min = Secretion Tm
Reabsorbed X = 100 µmoles/min = Reabsorption Tm
Urine X = 25 + 100 — 100 = 25 µmoles/min = Filtered X

Line c represents urinary excretion when reabsorption Tm = 100 µmoles/min, and secretion Tm = 0. Reabsorption Tm is reached at a plasma level of 1 µmole/ml. Urinary excretion then commences, and runs parallel to *line a*. This line corresponds to the "Excreted" line in fig. 1.

The difference between *line ba* and *line c* is that secretion Tm = 100 µmoles/min in *line ba* and 0 µmoles/min in *line c*. Reabsorption Tm = 100 µmoles/min

in both cases. Evidently *line c* can be reached (from *ba*) by gradually diminishing the secretion Tm. *Line a* will then move to the right. It will soon originate directly from the abscissa; this occurs when secretion Tm has diminished to 80 µmoles/min. It is evident that *line d* may represent

either	or
Reabsorption Tm = 100 µmoles/min Secretion Tm = 80 µmoles/min	Reabsorption Tm = 20 µmoles/min Secretion Tm = 0 µmoles/min

Thus tubular secretion of sulfate of considerable magnitude, cannot be excluded by the shape of the reabsorption curve.

Recent data, presented by BECKER and THOMPSON (1959) may be interpreted in terms of simultaneous secretion and reabsorption. The excretion of radiosulfate ($Na_2S^{35}O_4$), creatinine and radiopotassium (K^{42}) was studied after instantaneous injection into the renal artery. At normal plasma sulfate concentration (C_{SO_4} = 2 — 6 % of C_{Inulin}), the peak of S^{35} excretion occurred before the creatinine peak, but after the appearance of K^{42}. At an elevated plasma sulfate level (CSO_4 = 88 — 95 % of C_{Inulin}), the peaks of S^{35} and creatinine excretion occured together, but after the appearance of K^{42}. Becker and Thompson interpreted the early peak of S^{35} at normal plasma levels as "ion flux from peritubular to tubular fluid and the earlier K^{42} appearance is thought to be due to ion flux at a more distal site in the nephron".

Occurrence of simultaneous secretion and reabsorption by active transport might explain these results. This is illustrated by the following calculations:

I. *Assume:* Plasma SO_4 = 1 µmole/ml.
GFR = 100 ml/min.
RPF — GFR = 400 ml/min.
Reabsorption Tm = 495 µmoles/min.
Secretion Tm = 400 µmoles/min.

Then:

Filtered $SO_4 = 100 \times 1 = 100$ µmoles/min.
Secreted $SO_4 = 400 \times 1 = 400$ µmoles/min.
Reabsorbed SO_4 = 495 µmoles/min.

Urine $SO_4 = 500 - 495 = 5$ µmoles/min, of which $\frac{400}{100 + 400}$ = 4/5 derive from secretion.

(Note that C^{FOS} = 5 ml/min = 5 % of GFR).

II. *Assume:* Plasma SO_4 = 10 µmoles/min.
Other values as in I.

Then:

Filtered $SO_4 = 100 \times 10 = 1\,000$ µmoles/min.
Secreted $SO_4 = 400$ µmoles/min.
Reabsorbed $SO_4 = 495$ µmoles/min.

Urine $SO_4 = 1\,400 - 495 = 905$ µmoles/min, of which only $\frac{400}{1\,000 + 400} = 1/3.5$ derive from secretion.

(Note that C_{SO_4} = 90.5 ml/min = 90.5 % of GFR).

Thus, at low plasma levels, the major part of the sulfate in the urine could derive from tubular secretion. This could explain the early excretion peak of $S^{35}O_4$ in the experiments of Becker and Thompson.

Variation of sulfate reabsorption Tm in female dogs

LOTSPEICH (1947) presented data from two dogs. In one dog, the Tm was around 0.1 mmoles/min, in the other around 0.05 mmoles/min. These rates corresponded roughly to 0.19 and 0.12 mmoles/100 ml GF (glomerular filtrate), i.e. 0.19 or 0.12 mmoles sulfate were reabsorbed from 100 ml of glomerular filtrate. Thus there was a considerable difference between the two dogs. Later work has shown variation not only from dog to dog, but also between different experiments in the same dog. As the Tm has shown a tendency to vary with the glomerular filtration rate (BERGLUND and LOTSPEICH 1956 a), it has been found profitable to express the reabsorption in terms of µmoles/100 ml GF rather than µmoles/min.

The variability of the sulfate Tm is illustrated in tables 6—8. In all experiments included, sodium sulfate was given to raise the filtered load of sulfate above Tm levels. The reabsorption of sulfate (= sulfate Tm) was measured in 3—4 control periods; the mean of these was calculated in each experiment. — GFR was measured by the clearance of exogenous creatinine. Inorganic sulfate was measured by the method of POWER and WAKEFIELD (1938).

Most experiments were done on unanesthetized dogs. In two dogs, D and F, anesthesia was necessary, and sodium pentobarbital was used. This seemed to have no influence on the Tm. — Four dogs were excluded, because less than 4 experiments were done in each. Dogs "BIB" and "Helen", two deaf dalmatiar puppies of the same litter between 1/2 and 1 year of age, were treated as one dog in the statistical analysis.

Table 6. Sulfate Tm, average in dogs and cities

Dog	Weight	Number of exp:s	µmoles/min		µmoles/100 ml GF	
			Mean	Range	Mean	Range
1	19.5	18	69	52—89	116	75—154
2	20	36	153	81—238	155	110—190
3	21	13	117	39—159	189	134—249
4	19	5	80	31—123	126	53—188
5	15	11	76	51—103	164	144—187
6	22.5	12	167	102—218	174	136—239
7	18	5	92	64—136	172	153—201
8	21	11	70	30—148	93	50—187
Cinti		8 dogs	103		149	
A	15	5	118	80—243	184	134—281
B	?	6	85	59—103	136	116—167
C	18	13	100	55—145	149	95—208
D	17	8	99	62—133	164	133—194
E	23	18	155	68—276	184	118—317
F	26	5	106	90—122	140	130—150
G	13	11	92	64—183	165	110—334
BIB Helen	19 17.5	4	134	104—175	199	160—259
Boston		8 dogs	111		165	
U.S.A.		16 dogs	107		157	
J	14	4	115	93—156	202	173—236
K	18	6	216	149—301	233	174—297
M	19—33	7	131	84—150	187	158—214
Sthlm		3 dogs	154		207	
Total		19 dogs	114		165	

Table 7. Analysis of variance of sulfate reabsorption in terms of µmoles/min.

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Between cities	2	27 985	13 993
Among dogs	16	248 154	15 510
Experiments	179	184 020	1 028
Total	197	460 159	—
For cities: F = 13 993/16 606 = 0.84, d.f. 2, 16.1			

Method of calculation, cf section 9.11 (Subsampling in a completely randomized design), in Ostle, 1954.

F-test, cf section 9.13 (Satterthwaite's approximate test procedure in analyses of variance), in Ostle, 1954.

Dogs 1—14, A—C, "Helen" and "BIB", were in the recumbent positions, whereas dogs J, K and M were standing. The standing position is much preferable. Venous blood samples are easily obtained through an indwelling needle in the front leg. It is also possible to obtain arterial blood samples through a catheter, previously introduced by the method of SELDINGER (1953) with the dog in the recumbent position.

In table 6, the average Tm values for the dogs are tabulated, together with averages for each city. Analyses of variance were calculated on the tabulated data (tables 7 and 8). It is evident, by comparison of the two tables, that the variance component due to variation among dogs is higher for Tm in µmoles/min than for Tm in µmoles/100 ml GF. This might be due to two factors: 1. The Tm might vary with the GFR in individual dogs (BERGLUND and LOTSPEICH 1956 a); 2. Calculation of Tm in terms of µmoles/100 ml GF compensates for different sizes of dogs.

The average Tm (µmoles/100 ml GF) was higher in the Stockholm dogs than in the Boston and Cincinnati dogs (table 6). The variation between cities was not statistically significant (table 8), but was large enough for the investigation of possible causes. Differences in experimental conditions were considered. The position of the dogs seemed to be most important, as the dogs were standing up in Stockholm, but in a recumbent position in U.S.A. The effect of position on the sulfate Tm was therefore tested in two experiments (fig. 3). In these experiments, sulfate was analyzed by the method of BERGLUND and SÖRBO (1960). The results do not show any significant difference between the standing and recumbent positions. — Other possible experimental differences include factors such as experimental skill, care and diet of the dogs, and environmental temperature. None of these factors could be easily tested in acute experiments.

Table 8. Analysis of variance of sulfate reabsorption in terms of **µmoles/100 ml GF.**

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Between cities	2	52 358	26 179
Among dogs	16	122 521	7 658
Experiments	179	221 219	1 236
Total	197	396 098	—
For cities: $F = 26\ 179/8\ 084 = 3.24$, d.f. 2, 15.7. $0.10 > P > 0.05$			

Ref: See table 7.

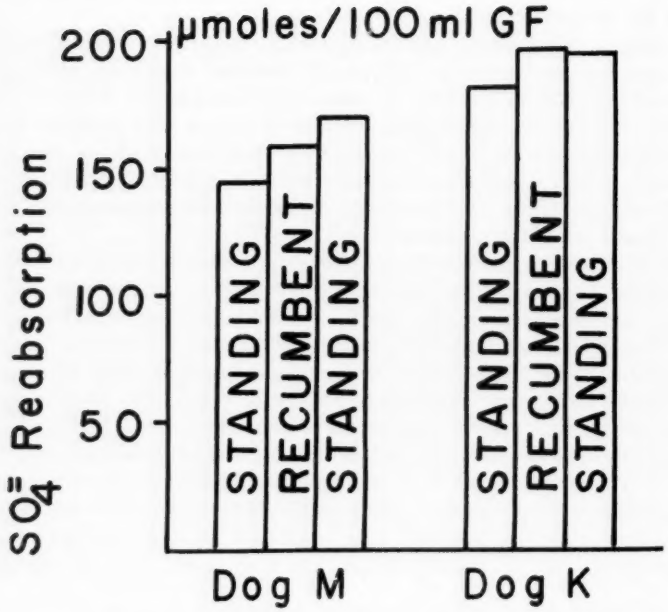

Fig. 3. Sulfate reabsorption Tm in two dogs in standing and recumbent positions. Each bar is average of 4 clearance periods.

Effects of other anions, glucose and amino acids, on sulfate reabsorption in the dog

Various inorganic salts, amino acids and other organic substances have been tested regarding their effect on sulfate reabsorption. A number of anions, amino acids and glucose depress the reabsorption, whereas phlorizin enhances reabsorption.

The sulfate-thiosulfate transport system. A detailed presentation of this system is found in the paper by BERGLUND, HELANDER and HOWE (1960).

Thiosulfate is both secreted and reabsorbed by the renal tubules; in the female dog and in man, the two processes (secretion and reabsorption) usually are of equal magnitude. The secretion of thiosulfate is inhibited by carinamide (BUCHT 1949, FOULKS et al. 1952). This makes the separate study of thiosulfate reabsorption possible.

Sulfate and thiosulfate mutually inhibit each others reabsorption in the tubules. The mutual inhibition is of the "competitive" type. This means that the relative reabsorption rates of sulfate and thiosulfate depend on the ratio between sulfate and thiosulfate in the plasma.

The reabsorption of thiosulfate was studied in separate experiments. At normal sulfate levels, reabsorption of thiosulfate increased in a semilogarithmic fashion with rising thiosulfate levels. The semilogarithmic curve was explained by increasing dominance of thiosulfate over sulfate in a competitive reabsorption system.

By using a Lineweaver — Burk plot, the thiosulfate Tm was estimated to be 65 % of sulfate Tm. Theoretical curves were then calculated for the reabsorption of sulfate and thiosulfate in each others presence. A comparison of experimental data with these curves led to the conclusion that the inhibition of sulfate reabsorption by thiosulfate was of "partially competitive" type, whereas sulfate inhibited thiosulfate in a "fully competitive" fashion.

Increasing sulfate levels in the plasma had no effect on the urinary excretion of thiosulfate, if carinamide was not present. Thus sulfate seemed to depress not only reabsorption, but also secretion of thiosulfate in the tubules. Secretion of sulfate could, however, not be demonstrated.

The relative sites of secretion and reabsorption of thiosulfate in the tubules were also studied. At low plasma levels, no thiosulfate appeared in the urine. This was explained by assuming that secretion occurred at a site proximal to reabsorption, or at the same level. Both filtered and secreted thiosulfate could then be reabsorbed. When the plasma level is raised, reabsorption becomes saturated, and thiosulfate then appears in the urine.

The results have been given the following interpretation: Sulfate and thio-

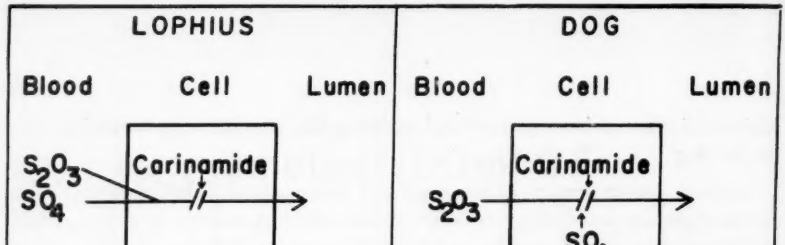

Fig 4. Transport of sulfate and thiosulfate, and effect of carinamide, in Lophius and in the dog. (From BERGLUND, HELANDER and HOWE 1960).

sulfate are reabsorbed through the tubule cells by an identical "carrier" mechanism. Thiosulfate shows higher affinity than sulfate to the carrier. This accounts for lower Tm but higher inhibiting power of thiosulfate. — Thiosulfate is secreted through the tubule cells by an identical or similar "carrier". Affinity between sulfate and the "thiosulfate secretion carrier" is indicated by the inhibition of thiosulfate secretion. — Similarities between dog and Lophius in respect to the renal tubular transport of sulfate and thiosulfate ions are summarized in fig. 4.

BERGLUND, HELANDER and HOWE (1960) also suggested that the direct renal effect of thiosulfate could explain the sulfaturia, that occurs after thiosulfate administration. This would invalidate earlier proof of oxidation of thiosulfate to sulfate *in vivo*. Experiments with S^{35}-marked thiosulfate do, however, show that such an oxidation occurs (SKARZYNSKI, SZCZEPKOWSKI and WEBER 1959). The two sulfur atoms were labelled alternatively, and sodium thiosulfate (1 gm $Na_2S_2O_3 \cdot 5\ H_2O$/kg body weight) was injected subcutaneously into rats. In 24 hours, about 25 per cent of the thiosulfate was excreted as such in the urine. Of the remaining thiosulfate, practically all (98 %) of the inner sulfur of the thiosulfate molecule, but only 40 per cent of the outer sulfur, was recovered in the urine as sulfate. Thus the two sulfur atoms of the thiosulfate molecule are oxidized to sulfate at different rates, and probably by different paths.

Effects of glucose and phlorizin. Glucose lowers the sulfate reabsorption Tm. When the reabsorption of glucose is abolished with phlorizin, the sulfate Tm rises above control values. The effects of glucose and phlorizin on the reabsorption of acetoacetate and phosphate are identical with the effects on sulfate

reabsorption (COHEN, BERGLUND and LOTSPEICH 1956). Evidently there is a common step in the reabsorption of glucose and the three anions (acetoacetate, phosphate and sulfate). Phlorizin abolishes the dominance of glucose in this system, probably by preventing the entrance of glucose into the cells of the renal tubules. Support to this theory is given by the work of KRANE and CRANE (1959), showing that phlorizin inhibits the entrance of D-galactose into slices af rabbit kidney cortex.

Interaction between acetoacetate, phosphate and sulfate. The demonstration of a common sensitivity to glucose and phlorizin led to an investigation of possible interaction between acetoacetate, phosphate and sulfate ions in the tubules (COHEN, BERGLUND and LOTSPEICH 1957). Acetoacetate lowered the Tm for phosphate and sulfate; phosphate lowered the Tm for sulfate only. Sulfate depressed neither acetoacetate Tm nor phosphate Tm. Thus the dominance of the three ions in tubular reabsorption was in the order acetoacetate > phosphate > sulfate, but the magnitude of the Tm:s was in the order acetoacetate > sulfate > phosphate. The lack of mutual inhibition, and the disagreement between transport rates and depressing action does not indicate competition for a common carrier site. The original paper suggested "net transport mechanisms which are in part separate for each of the three anions".

Effect of sodium chloride, thiocyanate and nitrate. LOTSPEICH (1947) demonstrated that sodium chloride lowered the sulfate Tm in dogs. After intravenous injection of approximately 0.1 gm NaCl/kg body weight, the sulfate Tm remains constant for about 30 minutes; during the next 20 minutes it gradually falls to a level around 2/3 of the control value (BERGLUND and LOTSPEICH 1956 a). In this respect the effect of sodium chloride differs from that of thiosulfate and of amino acids; full effect of sodium thiosulfate and of glycine is obtained within 15 minutes after intravenous injection.

Later experiments have shown that sodium chloride depresses sulfate reabsorption only when the sulfate level in the plasma is elevated above Tm levels. Thus sodium chloride does not cause any sulfaturia at normal sulfate levels. In this respect sodium chloride differs from sodium thiosulfate.

The reasons for the time-lag and dependence on the plasma sulfate level are not known. Two possible mechanisms are proposed. 1. The reabsorption mechanism for sodium and chloride is always saturated in the proximal tubules, but not in the distal tubules. Increased filtered load of sodium chloride will therefore affect only the distal tubules. Sodium or chloride ions, or both, might accumulate in the cells of the distal tubules, or in the surrounding interstitial fluid. Such an accumulation might, in turn, depress the sulfate reabsorption. This requires that part of the inorganic sulfate is reabsorbed in the distal tubules. 2. The intravenous injection of sodium chloride might depress sulfate Tm through some hormonal mechanism. — Neither one of these theories has been subjected to experimental tests.

Sodium thiocyanate and sodium nitrate have also been reported to depress the sulfate Tm in the dog (COHEN, BERGLUND and LOTSPEICH 1957). The experiments with sodium thiocyanate were done on two anesthetized dogs. The depression of sulfate Tm was similar to that caused by sodium chloride (tested in the same dogs), both with respect to magnitude and latency period. The effect of sodium thiocyanate was, however, not quite reproducible, and has not been repeated in the unanesthetized dog.

Effect of bicarbonate and ferrocyanide ions. Sodium bicarbonate caused a slight depression of the sulfate Tm in 9 experiments out of 10. Sodium ferrocyanide had no effect. It is therefore suggested that only those inorganic anions, which are themselves actively transported through the cells of the renal tubules, can depress the reabsorption of sulfate (BERGLUND 1960).

Effect of amino acids. A number of amino acids depress the sulfate Tm. Only the L-amino acids (which are also reabsorbed) have this effect (BERGLUND and LOTSPEICH 1956 b). The depressing effect of four amino acids fell in the same order as the magnitude of their own reabsorption Tm:s, namely L-alanine > glycine > L-glutamic acid > L-arginine. Thus the effect seems to be due, in some way, to the active transport of the amino acids in the renal tubules. It may be recalled that glycine did not affect the transport of sulfate in Lophius; neither was there any active transport of glycine in this species (BERGLUND and FORSTER 1958).

Effect of diet. In dogs transferred from a regular diet to a low protein diet, the plasma level of sulfate increased from an average of 1.6 µmoles/ml to 2.2 µmoles/ml (4 dogs), in spite of lower intake of sulfur (GERSHBERG, HODLER and GASCH 1954). Urinary excretion of sulfate decreased, and the reabsorption Tm for sulfate showed a 30—120 % increase, when measured in terms of µmoles/min. A connection between the low protein diet effect and the amino acid effect, referred to above, seemed possible, but plasma levels and excretion rates of amino acids were not measured.

Summary. The reabsorption of sulfate is depressed by a number of substances, which themselves are actively reabsorbed. These substances may be divided into three groups.

1. Thiosulfate. — Sulfate and thiosulfate show *mutual, "competitive"* or *"partially competitive" antagonism.* The Tm:s for the two ions are in the same order of magnitude. The conclusion seems justified, that thiosulfate and sulfate are transported through an identical carrier mechanism.

2. Acetoacetate, phosphate, nitrate, glucose, certain amino acids. — These substances depress sulfate Tm, but sulfate apparently has no effect on the transfer inhibitors. They seem to utilize separate transport mechanisms, which at one step or another affect the sulfate reabsorption. Whether the inhibition

of sulfate reabsorption is "competitive" or "non-competitive" has not been settled.

3. Sodium chloride (and sodium thiocyanate). — Delayed depression of the sulfate Tm, apparently only at elevated plasma levels of sulfate. The delayed depression makes it doubtful that sodium chloride acts by utilizing the same transport system as sulfate.

The ferrocyanide and bicarbonate ions, which probably do not enter the tubule cells, have no marked effect on the sulfate Tm.

Low protein diet raises the sulfate Tm in the dog. It is suspected that this is due to diminished amino acid load in the tubular urine.

Endocrine regulation of sulfate reabsorption

GERSHBERG, HODLER and GASCH (1954) showed that cortisone acetate (3—8 mg/kg/day for 6 days) increased reabsorption Tm for sulfate (expressed as μmoles/min) by approximately 60 % in two dogs.

Growth hormone also increased the sulfate Tm (GERSHBERG and GASCH 1956). Four dogs were given 3 mg/kg/day of growth hormone intramuscularly for 3 days. In 3 of the dogs sulfate Tm increased. If calculated in μmoles/100 ml GF, the increase was 133 %, 51 % and 16 % respectively. In one dog the Tm decreased by 21 %.

GERSHBERG, HEINEMANN and STUMPF (1957) measured renal functions in a patient with gigantism and acromegaly. GFR was 325 ml/min, and the Tm values for glucose and PAH were markedly elevated. Sulfate Tm was 289 μmoles/min; this corresponded to 89 μmoles/100 ml GF, compared with 30—76 μmoles/100 ml GF in a normal man. Unpublished studies by BECKER et al. (personal communication) indicate, however, that the value of 89 μmoles/100 ml GF is not above the normal range.

The scanty data reported so far thus indicate that cortisone and growth hormone increase sulfate Tm, but further documentation is desirable.

Sulfate reabsorption in man

BECKER et al. (personal communication) studied reabsorption of inorganic sulfate in 15 subjects without apparent renal disease. At increased filtered loads of sulfate, a Tm was reached. The Tm averaged 103 ± 57 μmoles/min (mean ± standard deviation), or 88 ± 43 μmoles/100 ml GF. Reabsorption Tm is therefore reached only when the plasma level has been raised to 0.88 μmoles/ml or higher, i.e. more than twice as high as the normal plasma level of 0.35 μmoles/ml (cf page 8). Also, significant amounts of sulfate appear in the urine at low plasma levels. The reabsorption of inorganic sulfate in man seems to be less effective below Tm levels than it is in the dog (cf fig. 1).

Plasma levels of inorganic sulfate in renal insufficiency

DENIS and HOBSON (1923) published data on the concentrations of several inorganic ions in sera from 21 patients with nephritis. The phosphate and sulfate levels were markedly elevated in more than half of the cases, sodium and chloride in four cases, whereas calcium was lowered in several cases. They noted that "from the percentage stand point probably the most striking change — — — is shown in the sulfate fraction". A value of 5 µmoles of sulfate/ml serum was reached in two cases.

DENIS (1923) also found markedly elevated serum sulfate levels (up to 8 µmoles/ml) in rabbits 2—3 days after subcutaneous administration of uranyl acetate (2—3 mg of the hydrated salt per kg body weight).

Later, DENIS and REED (1927) studied the sulfate concentration in blood of dogs after administration of uranyl acetate (route of administration not stated). In all dogs albuminuria appeared after the administration of uranyl acetate. A dose of 0.3 mg/kg body weight caused a diuresis (urine flow increased from 0.3 to 1.0 liter per 24 hours), lowered blood sulfate level, but no change in blood NPN (non-protein-nitrogen). After 0.4 mg/kg, and after 3 mg/kg, urine flow increased up to 2 liters per 24 hours, blood sulfate decreased and NPN increased. After 13.5 mg uranyl acetate per kg, urine flow decreased, whereas the levels of blood sulfate and NPN rose to extreme values.

Morphological evidence from rabbits (SUZUKI 1912) and dogs (HAYMAN et al. 1939) indicates that uranium mainly damages the distal portion of the proximal tubule, but there are also (with large doses) changes in the glomeruli. Probably, small doses of uranium depress reabsorption in the proximal tubules, thereby causing an osmotic diuresis and a fall of the blood sulfate level. After large doses of uranium, glomerular filtration rate falls (HAYMAN et al. 1939), which results in azotemia and high blood sulfate levels.

A number of investigators have confirmed the rise in serum inorganic sulfate levels in renal insufficiency. DENIS and HOBSON (1923) found rather parellel increases of the levels of serum inorganic sulfate, blood creatinine and serum NPN. MACY (1934) and ØLLGAARD (1937) showed that serum inorganic sulfate and blood urea increased in a parallel fashion in patients with chronic renal disease. BJERING and ØLLGAARD (1939) found marked elevation of the serum sulfate levels in man only when the exogenous creatinine clearance fell below 20 ml/min.

An elevated serum level of inorganic sulfate in renal insufficiency is most likely due to reduced glomerular filtration rate. As an indicator of renal insufficiency, the serum sulfate level might perhaps be as sensitive as the levels of serum creatinine, NPN or blood urea.

Uptake of inorganic sulfate by kidney slices *in vitro*

Using $Na_2S^{35}O_4$, DEYRUP and USSING (1955) showed that rat kidney slices, incubated *in vitro,* accumulate sulfate ions. This occurred in slices from renal cortex, but also to a considerable degree in slices from the outer region of the medulla. The uptake was reversed by CN^-, cooling to room temperature, and by interference with adequate oxygenation. It occurred optimally in the presence of 0.04 M K^+ in the medium, and decreased as the Na^+ concentration rose.

BERGLUND and DEYRUP (1956), also using rat kidney slices, measured the *in vitro* uptake of sulfate ions, by using $Na_2S^{35}O_4$ and by analysis with a benzidine method on trichloroacetic acid filtrates of slice homogenates. When the slices were incubated in KCl-sucrose, the two methods agreed. In one series the benzidine method showed a sulfate content of 1.98 ± 0.79* μmoles/gm tissue (initial wet weight) before incubation, and 3.70 ± 0.80* μmoles/gm after incubation, while the $S^{35}O_4$ analysis showed 3.63 ± 0.97* μmoles/gm after incubation. Thus there was a net uptake of sulfate in the slices, and the distribution of radiosulfate seemed to equal the distribution of benzidine precipitable sulfate.

In a NaCl-sucrose medium, the net uptake of sulfate was insignificant, as measured by the benzidine method (2.53 ± 0.84* μmoles/gm before incubation, 2.99 ± 0.62* μmoles/gm after incubation), and the $S^{35}O_4$ analysis after incubation showed low values (1.48 ± 0.22* μmoles/gm). The radiosulfate apparently was distributed in only half of the benzidine-precipitable sulfate of the slice.

This indicates the presence of two compartments in the slices. One compartment is freely available for the exchange of sulfate ions, but shows no net uptake. The second compartment is unavailable for exchange of sulfate ions in the NaCl-sucrose medium, but shows net uptake of sulfate in the KCl-sucrose medium.

Kidneys from various species were tested with respect to uptake of sulfate ions from the KCl-sucrose medium (DEYRUP 1956). The uptake was highest in slices from the rat. In guinea pig, dog and rabbit slices, the uptake was about 1/2, 1/3 and 1/4 as high as in rat kidney slices.

The subsequent studies were done on slices from rat renal cortex. Uptake was maximal between pH 6.0 and 7.0 of the medium, and decreased progressively as the pH rose from 7.2 to 8.0. Replacement of the 0.04 M KCl of the medium by 0.04 M KNO_3, or by a potassium phosphate buffer of similar strength, reduced the uptake by about 2/3. It may be recalled, that nitrate and phosphate also inhibit the reabsorption of sulfate *in vivo* (COHEN, BERGLUND and LOTSPEICH 1957).

* Footnote: Mean ± standard deviation. 6 determinations.

A number of other substances depressed the uptake of sulfate, namely glycine, glutamic acid and adenosine-triphosphate (ATP), m-aminophenol, diodrast, as well as a number of the tricarboxylic acid cycle intermediates. An almost complete inhibition was produced by 5×10^{-5} M dinitrophenol and by 1×10^{-3} M $HgCl_2$.

Phlorizin (4.5×10^{-4} or 4.5×10^{-3} M) produced up to 50 % enhancement of radiosulfate uptake. In the presence of monosaccharides, sulfate uptake was lower than in the controls, but could be restored to or above the control level by phlorizin. These effects of monosaccharides and phlorizin are strikingly similar to those produced by glucose and phlorizin on the *in vivo* transport in the dog (COHEN, BERGLUND and LOTSPEICH 1956).

The rate of loss of $S^{35}O_4$ after uptake from the KCl-sucrose medium was also studied by DEYRUP (1957). Cold (2° C) prevented the loss of $S^{35}O_4$ which always resulted from addition of metabolic inhibitors at 37° C. Deyrup suggested the presence of two processes in the slices: one involving uptake of $S^{35}O_4$, the second involving loss of $S^{35}O_4$, the latter being more temperature sensitive.

Numerous substances, which are secreted by the tubules *in vivo*, have been shown to accumulate in renal tissue *in vitro*. Of substances that are reabsorbed by the tubules, sulfate was the first one for which *in vitro* accumulation was demonstrated. It might be inferred that this accumulation is an *in vitro* expression of an *in vivo* secretion by the tubules, normally masked by a simultaneous reabsorption of larger magnitude. But the demonstration of galactose uptake by slices of rabbit kidney cortex (KRANE and CRANE 1959) makes this inference less valid.

On the mechanism of inorganic sulfate transport

The secretion of sulfate in the kidneys of marine fish, and the reabsorption of sulfate by the mammalian tubules, fulfill the following criteria of "active transport":

1. The transfer can take place against a concentration gradient. This is indicated by a urine/plasma concentration ratio for sulfate above 1.0 in marine fish, and a urine/plasma concentration ratio below 1.0 in dog and man.

2. The transport exhibits a transfer maximum, which, so-far, has been most well-defined in the dog.

3. Sulfate and thiosulfate, two ions with like structure, compete with each other in their transport through the renal tubule cells. The seemingly specific action of carinamide on the tubular secretion of these two ions also supports the idea of active transport.

An active transport may be conceived to take place by means of a "carrier" system. The sulfate-carrier complex might differ from the sulfate ion in the ability to penetrate the cell membrane. The "carrier" transport would involve at least three steps: 1. formation of a sulfate-carrier complex, 2. movement of this complex some distance through the cell membrane, 3. release of sulfate from the carrier. Alternatively, in step 2, the "carrier" might consist of stationary macro-molecules, and sulfate would move along the carrier through the membrane. Possibly, "active sulfate" (LIPMANN 1958) may be involved. "Active sulfate" is formed by a reaction between sulfate and two adenosine-triphosphate (ATP) molecules, giving rise to adenosine-3′-phosphate-5′-phosphosulfate. The "active sulfate" appears to be a general sulfate donor in biological reactions, e.g. in formation of phenol sulfates and steroid sulfate. An ester sulfate, of relatively small molecular size, would actually seem more likely than "active sulfate" as a diffusible sulfate carrier complex.

One might visualize the following chain of reactions in the transport of inorganic sulfate: 1. Formation of "active sulfate"; 2. Transfer of sulfate to an acceptor, which acts as a diffusible "carrier"; 3. Hydrolysis of the acceptor — sulfate complex.

The competitive inhibition, exerted by thiosulfate on sulfate transport by the tubules, can perhaps be explained by such a scheme. Thiosulfate has been shown to inhibit the formation of "active sulfate" by a competitive mechanism (HILZ, KITTLER and KNAPE 1959).

There is, at present, but little support for the proposed mechanism of sulfate transport. "Active sulfate" forming enzymes have been demonstrated in mammalian liver, but the kidney seems not to have been examined with respect to these enzymes. 3′, 5′-diphospho-adenosine (which is the part of "active

sulfate" remaining after transfer of sulfate to an acceptor) has, however, been found in rabbit kidney, in a concentration equal to 1/6 of that in the liver (GREGORY and LIPMANN 1957). Also, arylsulfatase has been demonstrated in mammalian kidney (RUTENBERG, COHEN and SELIGMAN 1952).

GENERAL SUMMARY

An account is given of present knowledge about the occurrence and characteristics of transport of inorganic sulfate by the renal tubules. Transport of sulfate ions occurs both among invertebrates (e.g. in the antennary glands of marine crustacea) and among vertebrates. Sulfate is secreted by the renal tubules of marine fish, and reabsorbed by the tubules in dog, man and rabbit. By secretion of sulfate, marine animals manage to reduce the concentration in the blood to levels less than 20 % of that in the sea. By reabsorption, on the other hand, mammals keep a relatively constant sulfate level in the plasma in face of varying intake of sulfur in the food.

In vivo transport of sulfate by the renal tubules has been studied mainly in the dog and in the aglomerular goosefish. In both species, the transport exhibits a "Tm", and is competitively inhibited by thiosulfate ions. Carinamide specifically inhibits the tubular secretion of both ions. Sulfate and thiosulfate are probably transported by identical pathways.

There are other interesting characteristics of sulfate reabsorption in the dog. It is inhibited by a number of anions, which are known to be reabsorbed as such by the tubules, but not by bicarbonate and ferrocyanide ions. The reabsorption is also depressed by amino acids; the higher the Tm of the single amino acid, the stronger is its effect on sulfate reabsorption.

Glucose depresses, and phlorizin enhances sulfate reabsorption. Similarly, *in vitro* uptake of inorganic sulfate by rat kidney slices is depressed by glucose and enhanced by phlorizin. The slices therefore seem to be a valuable tool in the study of sulfate transport. In the slices, sulfate occurs in two compartments, one of which is impermeable to sulfate in the absence of potassium in the medium.

A theoretical analysis of the reabsorption Tm curve in the dog has been presented. It is emphasized that simultaneous secretion of considerable magnitude cannot be excluded by the shape of the curve.

A mechanism has been proposed for sulfate transfer in the tubule cells, involving the formation of "active sulfate" from 2 ATP + $SO_4^=$, subsequent transsulfuration to a "carrier" of lower molecular weight, and final release of sulfate ions from the carrier.

The most pertinent literature on plasma levels of inorganic sulfate in man is reviewed. In patients with renal insufficiency the sulfate level is often markedly elevated, due to low glomerular filtration rate.

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